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Preferences of *Bactrocera cucurbitae* (Diptera: Tephritidae) to different colors: a quantitative investigation using virtual wavelength

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Abstract: Various colors have been used as visual cues to trap insect pests. For example, yellow traps for monitoring and controlling of the fruit flies have been used for a very long time. However, the chromatic cue of using color traps has never been quantitatively investigated. To reveal which wavelengths are most attractive to the fly, experiments were conducted to evaluate the preferences of *Bactrocera cucurbitae* to different colors. In this study, RGB values and virtual wavelengths were converted each other based on Dan Bruton' s wavelength to RGB function. Eight color papers were printed by color printer based on RGB values [(0, 213, 255), (0, 255, 146), (54, 255, 0), (129, 255, 0), (195, 255, 0), (255, 255, 0), (255, 190, 0) and (255, 119, 0)] and were chosen to test the color preference of the melon fly, *Bactrocera cucurbitae* in an octagon maze, and their equivalent wavelengths were 480 nm, 500 nm, 520 nm, 540 nm, 560 nm, 580 nm, 560 nm, and 600 nm, respectively. The color papers with the spectrum between 520 -560 nm showed higher attractiveness than the others, and that with the spectrum of 540 nm (chartreuse green, with RGB values of 129, 255, 0) showed the highest attractive rate. In addition, field preference experiment was done to confirm the effect of the chartreuse green color. The results suggest that chartreuse green stimuli (spectrum 540 nm) should be a crucial cue to attract *B. cucurbitae*, and chartreuse green sticky traps could be a useful device for monitoring and managing *B. cucurbitae*.

Key words: *Bactrocera cucurbitae*; color preference; RGB value; virtual wavelength; sticky trap

1 INTRODUCTION

The melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is distributed widely in Africa, Asia, Oceania, North America, and South America (Dhillon *et al.*, 2005), especially occurred in numerous provinces and regions in China (Liang *et al.*, 1989). It has been reported to damage more than 125 host plant species and is a major pest of cucurbitaceous vegetables, particularly the bitter gourd (*Momordica charantia*), muskmelon (*Cucumis melo*), snap melon (*C. melo* var. *momordica*), and snake gourd (*Trichosanthes anguina* and *T. cucumeria*) (Dhillon *et al.*, 2005). The melon fruit fly damage fruits by laying eggs in fruit tissues. The larvae feed inside the fruits and cause the fruits subsequently to rot or become distorted.

Color preference has been used for pest monitoring and controlling for a long time. For example, yellow traps have been applied in prevention and control of pests including whitefly, fruit flies, thrips, and leafminers. However, the chromatic cue of using yellow traps has never been quantitatively investigated. Although color preference has been intensively studied in insects including *Lygus* spp. (Blackmer *et al.*, 2008), *Liriomyza trifolii* (Parrella and Jones, 1985), *Thrips palmi* (Babu *et al.*, 2004), *Frankliniella occidentalis* (Hoddle *et al.*, 2002), *Franklinothrips orizabensis* (Hoddle *et al.*, 2002), whiteflies (Berlinger, 1980), fruit flies (Ravikumar and Viraktamath, 2007; Wu *et al.*, 2007), *Caliothrips fasciatus* (Harman *et al.*, 2007), and *Chaetocnema pulicaria* (Esker *et al.*, 2004), unfortunately, the colors used in these studies were only described qualitatively, such as amber yellow, yellowish brown, apricot, emerald green, reddish orange, *etc.* Only a few studies utilized spectrometer to measure the reflectance spectrum of the color sticky card (Ranamukhaarachchi and Wickramarachchi, 2007; Wu *et al.*, 2007; Blackmer *et al.*, 2008), but the selected most effective color can not be precisely

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reproduced, which severely affected its operability.

The current color models include RGB (red, green, blue), CMYK (cyan, magenta, yellow, black), Lab (luminosity, a channel – green/magenta, b channel – blue/yellow), HSV (hue, saturation, value), HSL (hue, saturation, intensity) and NCS (natural color system). The RGB color model is an additive color model, in which red, green and blue lights are added together in various ways to reproduce a broad array of colors. Besides conventional photography, RGB model is mainly used for sensing, representation and display of images in electronic systems including televisions and computers. The theoretical explanation of RGB model has been established before the electronic age based on human color perception, by which, RGB values can be converted to virtual wavelength (λ) through Dan Bruton's wavelength to RGB function, and *vice versa*. Unlike the RGB color model, Lab color is designed to approximate human vision. The Lab color space is used, *e. g.*, in Adobe Photoshop when graphics for print have to be converted from RGB to CMYK, as the Lab gamut includes both the RGB and CMYK gamut. Also it is used as an interchange format between different devices as for its device independency. In this study, RGB values were quantitatively converted into virtual wavelengths, which provide a feasible method to set optimal color parameters for trapping *B. cucurbitae*.

2 MATERIAL AND METHODS

2.1 Laboratory study

Fruit flies were obtained from a laboratory colony maintained at room temperature, the colony was reared on pumpkin fruits, and the 5th – 6th day old adults were used for test and fed with a yeast-sucrose mixture (1∶3 by weight). Different RGB values were converted correspondingly to virtual wavelengths based on Dan Bruton's wavelength to RGB (www.physics.sfasu.edu/astro/color.html) function using computers (Table 1) and printed using a color inkjet printer (Hewlett Packard 5000). RGB values were converted to Lab values in Adobe Photoshop before printing. The test device was an improved octagon maze made of black polythene foam board (formed by surface film lamination with features of no easy distortion, easy processing) as previously described (Wu *et al.*, 2007). Chambers were separated from each other and the tested color papers were pasted on the outer surface of each chamber. The top of the choice room was covered with transparent film, with a hole in its center for releasing the insects (Fig. 1). The fruit flies restricted in a transparent container were put at the center hole of the transparent film. Container was opened and flies were allowed to fly freely. After 8 – 10 min, the number of *B. cucurbitae* distributed in each chamber was counted. The number of tested *B. cucurbitae* was not less than 30 in each experiment. Females and males were independently tested for more than 50 times. To avoid any impact of polarization from incident light on color preference of *B. cucurbitae*, octagon maze was placed under a shaded and cool condition. Its position was rotated after each test.

Table 1 List of colors used in laboratory tests and their RGB values and virtual wavelengths

RGB values	Color	Virtual wavelength (nm)
0, 213, 255	🟦	480
0, 255, 146	🟩	500
54, 255, 0	🟩	520
129, 255, 0	🟩	540
195, 255, 0	🟩	560
255, 255, 0	🟨	580
255, 190, 0	🟧	600
255, 119, 0	🟧	620

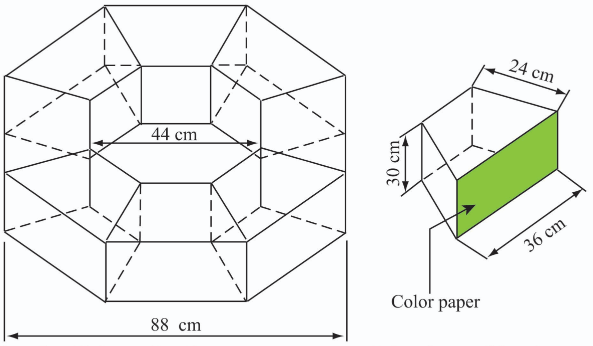

Fig. 1 Schematic of octagon maze used for the multi-color test

2.2 Field study

Eight colors used in the field study were printed on adhesive photo papers with the size of 30 cm × 40 cm using a color inkjet printer (Hewlett Packard 5000). Both sides of these color papers were evenly coated with pest-adhesive gel (Pherobio Technology Co. Ltd., China) and pasted on the KT cards with the thickness of 6 mm. The experiment was conducted on a sunny day in a bitter gourd field at Jinyin Experiment Station, Guangdong Academy of Agricultural Sciences, Guangzhou, China. The plants were irrigated and fertilized according to standard recommendations and not sprayed with any insecticide during the experimental period. Completely randomized block design was used and each block repeated five times (area of bitter gourd field is 533.6 m^2, divided into five area groups). The sticky cards were hung on the middle of bitter gourd scaffolding at 8:20 am. The numbers of female and male adults of *B. cucurbitae* trapped on each color paper were counted 24 h later.

2.3 Data analysis

The color preference of *B. cucurbitae* to certain color in the laboratory study was evaluated by attractive rate (AR) as calculated using the following formula:

AR = number of insects trend to certain wavelength/number of tested insects.

The differences in AR of different colors to *B. cucurbitae* were analyzed with "General Linear Model" of SPSS11.0 software. Data were arcsine transformed prior to analysis of variance and means were compared by Duncan's multiple range test. Parabolic equation $AR = a + b\lambda + c\lambda^2$ was iteratively fitted using the sequential quadratic programming method in SPSS 11.0 and the vertex coordinates were calculates as $[-b/2c, (4ac - b^2)/2c]$.

The difference in numbers of trapped *B. cucurbitae* adults in the field study on the color paper was analyzed using General Linear Model in SPSS11.0 software.

3 RESULTS

3.1 Relationship between attractive rate and virtual wavelength in laboratory study

The laboratory study showed that, among the eight selected colors, the attractive rates of colors with the wavelengths between 520 and 560 nm to *B. cucurbitae* adults were significantly higher than those of other colors (female: $F = 69.073$, $df = 7$, $P < 0.001$; male: $F = 47.296$, $df = 7$, $P < 0.001$) (Table 2).

The mathematical models of the relationship between attractive rates of different colors to female (AR_F) and male adults (AR_M) of *B. cucurbitae* and corresponding wavelength are parabola opening downwards.

$AR_F = -7.381 + 0.028\lambda - 0.000026\lambda^2$ ($R = 0.910$, $P < 0.05$);

$AR_M = -5.892 + 0.026\lambda - 0.000024\lambda^2$ ($R = 0.924$, $P < 0.01$).

Table 2 Attractiveness of different color papers to *Bactrocera cucurbitae* adults

Virtual wavelength (nm)	Female	Male
480	0.0793 ±0.005 e	0.0913 ±0.007 d
500	0.0987 ±0.007 d	0.1133 ±0.007 c
520	0.1920 ±0.007 a	0.1653 ±0.008 a
540	0.1747 ±0.006 b	0.1707 ±0.006 b
560	0.1893 ±0.006 ab	0.1993 ±0.009 b
580	0.1173 ±0.005 c	0.1220 ±0.008 c
600	0.0607 ±0.005 f	0.0560 ±0.004 e
620	0.0440 ±0.004 g	0.0400 ±0.004 e

Data in the table are mean ± SE, and different letters within the same column following the means indicate significant difference ($P < 0.05$) among the arcsine (x) transformed data analyzed by using GLM procedure and Duncan's mean separation test.

The calculated vertex coordinates (λ, AR_{max}) of mathematical models for female adults of *B. cucurbitae* were (538.4615, 0.1575) and those for male adults were (541.6667, 0.1497). In other words, both male and female adults of *B. cucurbitae* had maximum AR at the wavelength of 540 nm, which was corresponding to the RGB value of (129, 255, 0). This color is luminous vivid chartreuse green (http://www.perbang.dk/rgb/81FF00/). By inputting its parameter into computer system (Microsoft Word or Photoshop software), it can be authentically printed out.

3.2 Color preference of the melon fly in filed study

Field study also showed that color traps with the wavelength of ~540 nm were more attractive to female adults of *B. cucurbitae* than those with other wavelengths ($F = 33.987$, $df = 8$, $P = 0.000$).

The mathematical models of the relationship between average number captured per card (Y) and corresponding wavelength are parabola opening downwards.

$Y_{Female} = -171.348 + 0.656\lambda - 0.00060\lambda^2$ ($R = 0.736$, $P < 0.05$);

$Y_{Male} = -252.712 + 0.944\lambda - 0.00086\lambda^2$ ($R = 0.912$, $P < 0.01$).

The calculated vertex coordinates (λ, Y_{max}) of mathematical models for female adults of *B. cucurbitae* were (545.5846, 11.5461) and those for male adults were (546.8277, 10.7425).

The numbers of female adults of *B. cucurbitae* attracted on other color traps were not significantly different. The number of male adults of *B. cucurbitae* attracted on color traps with the wavelength of 540 nm was significantly higher than those on other color traps (female: $F = 8.027$, $df = 8$, $P < 0.001$, male: $F = 11.082$, $df = 8$, $P < 0.001$). The numbers of male adults of *B. cucurbitae* attracted by color traps with the wavelengths of 500, 520, 540 and 560 nm, or 500, 520, 560, 580 and 600 nm were not significantly different (Fig. 2).

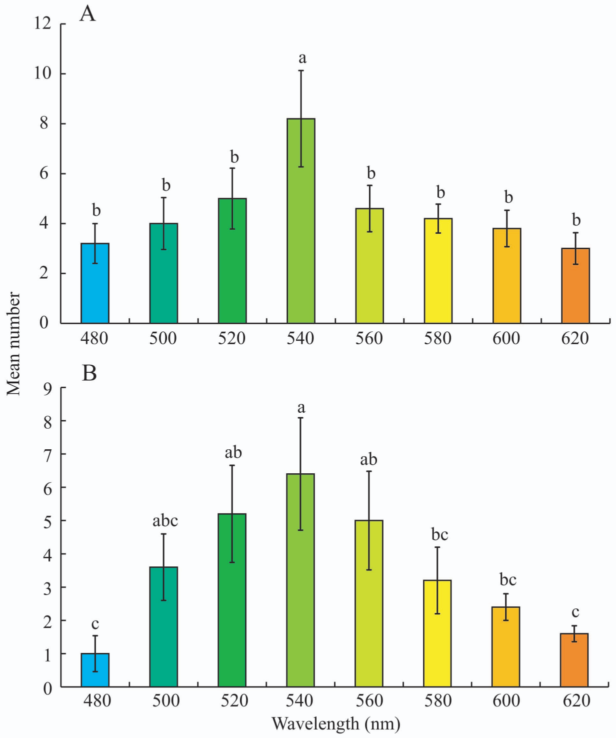

Fig. 2 Number of female (A) and male (B) adults of *Bactrocera cucurbitae* caught by different color sticky traps used in bitter gourd field

Data in the figure are mean ± *SE*, and different letters above bars indicate significant difference ($P < 0.05$) analyzed by using GLM procedure and Duncan's mean separation test.

4 DISCUSSION

Color is a wonderful combination of physics and human perception. There is no unique one-to-one mapping between wavelength and RGB values. The values of wavelength should only be used as approximate colors, for example, in false color schemes (Http://www.efg2.com/Lab/ScienceAndEngineering/Spectra.htm). Some researchers utilized substrates that reflect maximally to explore the wavelength of sticky trap (Ranamukhaarachchi and Wickramarachchi, 2007; Wu *et al*., 2007; Blackmer *et al*., 2008), but they failed to reciprocally convert color parameters to wavelength. This study shows that quantitative and reciprocal conversion between RGB values and virtual wavelengths is feasible by using Dan Bruton's wavelength to RGB function.

Many phytophagous insects respond positively to light reflectance patterns of their host plants, especially the generalist herbivore, and these responses can be quite specific. Very often, color spectra between 500 and 580 nm elicit the strongest response; however, in some cases, the response may be associated with a particular stage of the host plant (*e. g*., flowering or fruiting stage) when reflectance is maximal in other regions of the spectrum (Prokopy and Owens, 1983). It has been speculated that whitefly *Bemisia tabaci* (Atakan and Canhilal, 2004), aphids (Park *et al*., 2001), fruit flies (Robacker and Heath, 2001) and leafminers such as *Liriomyza trifolii* (Ozawa *et al*., 1999) prefer yellow, while thrips such as *Frankliniella occidentalis* prefer blue (Chen *et al*., 2004) or white (Hoddle *et al*., 2002). Therefore yellow or blue sticky traps have been widely used. However, recent studies have shown that some insects such as the oriental fruit fly *Bactrocera dorsalis* prefer green (spectra around 500 – 570 nm) (Wu *et al*., 2007) rather than yellow (spectra around 580 nm).

Many insects are sensitive to both UV and yellow-green color (Agee 1985; Wu *et al*., 2007). For example, *Aleurodicus dispersus* is most sensitive to purple (405 nm) close to UV, followed by chartreuse (520 – 570 nm) (Zheng *et al*., 2010). However, Matteson and Terry (1992) reported that highly UV reflective white (78% reflectance at 365 nm) captured fewer thrips *F. occidentalis* than low UV reflective white (14% reflectance at 365 nm). Cho *et al*. (1995) also found that UV reflective white (66% reflectance at 365 nm) is less attractive to *Frankliniella* spp. than low UV reflective yellow and blue (35% reflectance at 365 nm). These data suggest that insects have different responses to self-luminous color traps and reflective color traps. The underlying causes for this phenomenon need to be further studied.

Color preference was similar between the sexes, and has been reported in other tephritid fruit flies, for example *B. dorsalis* (Wu *et al*. 2007). Probably, both sexes of *B. cucurbitae* use the same visual cues to detect a host plant that serves as mating sites as well as habitat and feeding sites. Further studies are needed to determine the relationship between visual cues and behaviors in *B. cucurbitae*.

In addition to *B. cucurbitae*, the chartreuse cards also trap some flies, bees, butterflies and ladybirds, indicating that some insects from orders Diptera, Hymenoptera, Lepidoptera and Coleoptera also have chartreuse tropism to some extent.

Therefore, chartreuse cards can also be used to trap and kill some natural enemies of insects.

In conclusion, this study showed that: 1) *B. cucurbitae* exhibited a broad response to hue with the spectra between 520 and 580 nm as predicted for generalist herbivore (Prokopy *et al.*, 1982; Prokopy and Owens, 1983) and chartreuse green is most attractive to *B. cucurbitae*; 2) RGB with parameter of (129, 255, 0) in decimal number system and 81FF00 in hex number system is the most attractive RGB color to *B. cucurbitae*. It can be converted to CMYK (46%, 0, 90%, 0), HSB (90°, 100%, 100%), and Lab (90, −61, 84). These parameters can be applied to produce paper trap cards (printed or printing), plastic trap plates and plastic bottles combined with lure odors. Despite the limitations that such sticky traps have (*e. g.*, they can only measure adult activity and are harmful to some beneficial insects), this information will be useful in monitoring adult *B. cucurbitae*.

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瓜实蝇对虚拟波长下不同颜色的趋性

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摘要： 利用害虫对不同颜色的趋性进行害虫防治，如利用黄板对实蝇的监测和防治已有很长的历史，然而尚未见把颜色量化进行实蝇对颜色偏嗜性研究的报道。为探明对瓜实蝇 *Bactrocera cucurbitae* 最具吸引力的颜色及其虚拟波长，本试验应用 Dan Bruton 的虚拟波长与 RGB 值的函数关系，把 RGB 值转换为虚拟波长；选择 RGB 值[(0，213，255)，(0，255，146)，(54，255，0)，(129，255，0)，(195，255，0)，(255，255，0)，(255，190，0)和(255，119，0)]的颜色进行打印，这些颜色对应的虚拟波长分别是480，500，520，540，560，580，560和600 nm；在八面体内进行瓜实蝇对8种颜色的偏嗜性试验。结果表明：波长在520～560 nm之间对应的颜色对瓜实蝇的吸引率高于其他虚拟波长对应的颜色，而540 nm(黄绿色，RGB值为129，255，0)对应的颜色纸对瓜实蝇的吸引率最大。此外田间颜色偏嗜性试验也证实了黄绿色对瓜实蝇有最强的引诱作用。结果说明，黄绿色(虚拟波长540 nm)是吸引瓜实蝇的关键颜色，黄绿色粘虫板可作为监测与防治瓜实蝇的一种有效方法。

关键词： 瓜实蝇；趋色性；RGB值；虚拟波长；粘虫板

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